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The Structure of Theiler's Virus Complexed with its Receptor

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Introduction

Multiple sclerosis (MS) is a serious human demyelination disease. It has long been speculated that virus infection may be a possible cause of MS. After years of study, it is clear that the mechanism of MS is more complicated than a simple infectious disease. Many factors which cause immune disorders eventually lead to the disease. However, virus induced demyelinating diseases are similar to those in MS. Theiler's murine encephalomyelitis virus (TMEV) infection in mice provides a valuable model for elucidating the molecular basis of viral neurovirulence related to demyelination. TMEV, which have been classified in the cardiovirus genus of the family *Picornaviridae* along with encephalomyocarditis virus and Mengovirus, can be divided into two groups based on neurovirulence characteristics after intracerebral inoculation of mice: (i) the highly virulent viruses represented by GDVII, FA and Ask-1, which cause a rapidly fatal encephalitis, and (ii) the less virulent viruses [also referred to as the TO (Theiler's original) group], such as BeAn and DA, which produce a persistent central nervous system (CNS) infection and inflammatory demyelination. Recombinant TMEV, constructed by exchanging corresponding genomic regions between the highly virulent GDVII and less virulent BeAn or DA virus cDNAs, have been used to map the determinant for virus persistence (and demyelination) to the capsid proteins; however, conflicting results regarding whether this determinant can include GDVII sequences have been reported[1].

In order to define the persistent determinants more clearly, we took the approach of combining structural analysis by X-ray crystallography with genetic and virological experiments. The structures of BeAn and DA visuses, the persistent strains, and GDVII virus, the highly neurovirulent and nonpersistent strain, have been solved by X-ray crystallography [2-4]. Overall, the architectures of BeAn, Da and GDVII viruses are very similar to that of other picornaviruses whose structures have been determined. Sequence alignment of the three major capsid proteins of these two groups of TMEVs showed that the amino acids that are identical in BeAn and DA viruses but are different in GDVII were clustered on the outside of the capsid in the loops and corners connecting the b strands. The amino acid changes between GDVII and BeAn capsid proteins were divided into conservative and nonconservative changes by Luo et. al. [2].

Results and Discussion

I. Structural Differences between Persistent & Nonpersistent TMEVs

Structural differences among native viruses. The differences between BeAn and GDVII were not dramatic. For example, the root mean square (rms) deviation between the Ca coordinates of the two viruses was 0.71 Å for 758 equivalent residues in VP1, VP2 and VP3. Five residues (with unidentified amino acid sequence) of the VP4 C-terminus are disordered in the GDVII, but ordered in the BeAn virus structure, whereas residues 1260-1263 and 1266-1276 of the VP1 C-terminus are ordered in the GDVII, but not in the BeAn virus structure. The ordered VP1 C-terminus forms a hook near the 3-fold axis, on top of the VP3 knob. A hydrogen bond between the carbonyl oxygen of VP1 Asp1267 and the side chain of VP3 Ser3058 appears to stabilize the VP1 C-terminus. The differences between DA and GDVII were compared to those between BeAn and GDVII; the rms deviation between the Ca coordinates of DA and GDVII viruses was 0.78 Å for 741 equivalent residues in VP1, VP2 and VP3.

Despite the overall structural similarity between GDVII and the two less virulent viruses, three sites of the GDVII structure showed local differences, mainly involving side chains. Residues 3059-3060 of VP3 have an rms deviation of Ca positions larger than 1.5 Å. This site is on the knob inserted in the ℬB strand of VP3 near the 3-fold axis. The side chain of Ser3058 forms a hydrogen bond with the carbonyl

oxygen of Asp1267 and conformation of residues 3058-3061 is consistent with a type I turn. In the BeAn and DA viruses, residues 3058 and 3060 are both Asn. No hydrogen bond is found between these two residues and the conformation does not resemble a type I turn. Residues 2170-2173 of VP2 and on PuffB is another site with an rms deviation of Ca positions larger than 1.5 Å. In the BeAn virus, the carboxyl group of Asp2170 forms a hydrogen bond with the side chain of Arg2172 and the side chain of Trp1095. The latter is probably mediated by a water molecule. In the DA virus, the carboxyl group of Asp2170 forms a hydrogen bond with the side chain of Arg2168. The side chain of Asp2170 in these two less virulent viruses in approximately at the same location. In the GDVII virus, Arg2172 is replaced by a Gln that forms a hydrogen bond with the side chain of Arg2168. Consequently, the side chain of Asp2170 is also relocated although this residue is the same in both the BeAn and GDVII sequences. The side chain of Arg2171 in the GDVII virus replaces a Ser in the BeAn virus and points to the solvent, above 90° to the side chain of Arg2172 in the BeAn virus. Such a rearrangement leads to the relocation of two charges. The third site is at the top of the loop II region. In the DA virus, there is a two-residue deletion after 1101 relative to other TMEV strains, and therefore, the loop II in GDVII and BeAn viruses is slightly larger that in DA virus.

The major differences which may be biologically relevant are two clusters of unconserved amino acid changes (cluster A & B) on the virus surfaces. Cluster A is around the third corner of VP1, while cluster B is located at the VP2 puff B. Although the main chain structure is similar in the cluster B region of all known TMEV structures, the side chains including an Arg have very different orientations. In BeAn virus, the carboxyl group of Asp2170 forms a hydrogen bond with the side chain of Arg2172 and side chain of Trp1095. The latter is probably mediated by a water molecule. In the DA virus, the carboxyl group of Asp2170 forms a hydrogen bond with the side chain of Arg2168. In both BeAn and DA viruses, the side chain of Asp2170 is in approximately the same direction and the side chain of Arg2172 is pointing to the solvent towards VP1 in the north-eastern direction. In GDVII, however, Arg2172 is replaced by a Gln that forms a hydrogen bond with the side chain of Arg2168. As a consequence, the side chain of Asp2170 is also relocated although Asp2170 is the same amino acid residue for all three viruses. In addition, the side chain of Arg2171 in GDVII virus replaces Ser2171 in both BeAn and DA viruses and points to the solvent in the north-western direction, about 90° to the side chain of Arg2172 in both BeAn and DA viruses. Such a rearrangement leads to the relocation of two charges. Further more, variant residues between virus groups are distributed around a gap between VP1 and VP2 surface loops. These residues are 1060-1063, 1076-1086, 1093-1097 and 1109 from VP1 and 2169-2172 from VP2 (Fig. 2). Variant residues 1076-1077, 1079, 1084, and 1093-1096 from VP1 are not exposed on the capside surface. However, these unexposed residues could influence the conformation of the exposed surface residues through direct or long-distance interactions. This gap has a very similar shape and charge distribution in BeAn and DA viruses, but quite different in GDVII virus. It is interesting to note that although Glu1060 is a conserved amino acid and the main chain structures are very similar in these two groups of viruses, the side chain orientation of Glu1060 is dramatically different in these two groups of viruses. In the less virulent and persistent native BeAn and DA viruses, the side chain of Glu1060 points toward the gap in a direction perpendicular to the radial axis. In the highly virulent and non-persistent native GDVII virus, the side chain of Glu1060 points to the solvent in a direction roughly parallel to the radial axis. A similar situation was observed for the side chain of residue 1086. In BeAn and DA viruses, 1086 is a Lys and its side chain points into the gap forming a salt bridge with Glu1060 side chain. In GDVII virus, Lys1086 is replaced by an Arg and its side chain points away from the gap toward the solvent in the radial direction, and as a result there is an opening between residue Glu1060 and Arg1086.

Putative Receptor Binding Site. When the structure of the BeAn virus was first determined, we postulated that a surface depression between VP1 and VP3 might be the site of interaction with the host receptor. To further define the receptor binding site, the structure of GDVII virus was superimposed on that of human rhinovirus 14 (HRV14). Except for the loops in VP1, the structure of TMEV closely resembles that of HRV14, especially the VP2 puffB and VP3 GH loop. Recently, the major group HRV residues involved in binding to its receptor, ICAM-I,

were mapped by cryoelectron microscopy in HRV16 complexed with ICAM-I. The VP1 loops I and II of the GDVII virus are not present in HRV. There would be spatial overlaps (steric hindrance) if the receptor of TMEV has to take the same position as ICAM-I. The TMEV receptor would have to move toward the VP3 GH loop if its receptor binds to this region and would probably make contact with VP1 loop I. The VP3 GH loop has been implicated in receptor binding of another cardiovirus, Mengo virus, by studies showing that high pH induces in the presence of phosphate conformational change in the VP3 Gh loop which can prevent Mengo virus from binding to its receptor. Note that the VP3 GH loop in DA virus is also disordered under the crystallization conditions. The conformation of the BeAn VP3 Gh loop is the same as in the receptor-binding form of Mengo virus. This further suggests that the receptor binding site is near the VP3 GH loop, as shown by the superposition of TMEV with the ICAM-I binding site of HRV14.

II. The Role of Sialyloligosaccharide Binding in Theiler's Virus Persistence

Structural Implications. As shown in the previous section, the gap formed by residues from both VP1 andVP2 was shaped differently in these two phenotypically different groups of viruses. The virus surface shows clearly that this gap is right next to the putative receptor binding site near the VP3 GH loop. Early studies on the attachment of GDVII and BeAn viruses to intact BHK-21 cells showed that these two groups of viruses share a common receptor but bind to the receptor differently. GDVII and BeAn viruses compete for the receptor with each other, but virus attachment was inhibited by neuramindase desialylation only to BeAn virus, not GDVII virus. This suggests that sialyloligosaccharides maybe part of the cellular receptor for the TO viruses. The proximity of the gap to the putative receptor binding site and the consistent correlation between the shape of the gap and virus interactions with sialyloligosaccharides on the host receptor. Amino acid sequence alignment of two groups of TMEV viruses, including neurovirulent GDVII and FA strains and persistent BeAn, DA, TO4 and Yale strains, shows that key amino acids in the gap region (phe1076, Lys1086, Ser2171, Arg2172 and Thr2173) are absolutely conserved within the persistent subgroup but different from those in the nonpersistent subgroup.

Block of virus attachment by sialyloligosaccharides. Based on the structural differences and the result of early experiments by Fotialis et al. [5] showing a reduction of virus attachment after neuraminidase treatment, we suggested that sialyllactose, the first three sugar molecules of unbranched sialylated oligosaccharides, is the receptor fragment of persistent strains of TMEV. Experiments were designed to exam the effects of sialyllactose on BeAn virus binding to BHK-21 cells. The data showed (Fig. 3) that the attachment of [^{35}S]-labeled BeAn virus to BHK-21 cells was reduced 75% and 90% by 5.0 mM and 9.0 mM of sialyllactose, respectively. Non-radiolabeled BeAn virus was shown to complete with the binding of radiolabeled BeAn virus. To eliminate the possibility that sialyllactose may affect BeAn virus binding by altering host cell surface, [^{35}S]-labeled BeAn virus binding was measured using BHK-21 cells pretreated with 9.0 mM sialyllactose followed by 3 washes of cooled PBS. There was no detectable change in binding of [^{35}S]-labeled BeAn virus (data not shown). This data provided evidence that sialyllactose is the receptor fragment for BeAn virus and can block its attachment to the host cell.

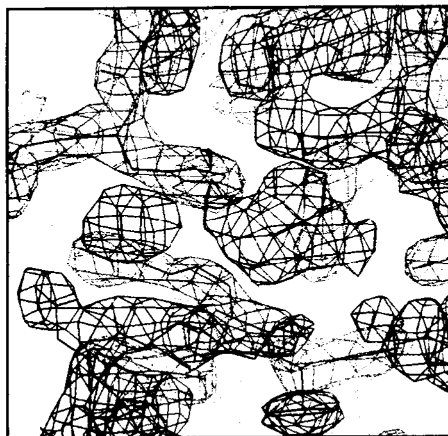
The role of sialyloligosaccharide binding. To test whether sialyloligosaccarides affect the replication of TMEVs in BHK-21 cells, virus infection in the presence of different concentrations of sialyllactose was carried out for both BeAn and GDVII. 30 hours after infection, BeAn was shown to be able to infect and induce cytopathic effect (CPE) in BHK-21 cells in the presence of 0.0 mM, 1.1 mM and 2.3 mM sialyllactose, while 5.4 mM and 9.0 mM sialyllactose can prevent CPE (Fig. 4). In the presence of 3.6 mM sialyllactose, plaques were observed. The virus yield was titered by plaque assay on BHK-21 cells. The titered virus yield was reduced by roughly 10 to 100 fold at each increment of sialyllactose concentration, with 10^5~10^6 reduction at 9.0 mM. Based on the chemical properties of sialyllactose and the result by Fotiadis et al. [5], it is likely that sialyllactose inhibits BeAn infection by blocking its cellular attachment. The different cellular changes caused by BeAn infection in the presence of sialyllactose at various doses indicated that sialyllactose does indeed block BeAn infection in BHK-21 cells, and the inhibition is does dependent. The dissociation constant of sialyllactose to BeAn is thus estimated to be between 3.6 mM to 5.4 mM. In contrast, CPE was observed in all the GDVII infections in the presence of 0.0

mM, 4.5 mM and 9.0 mM sialyllactose (data not shown). There was no difference in titered virus yield from each infection. This result supports our hypothesis that the different viral supports our hypothesis that the different viral surface features of BeAn and GDVII, such as the proposed gap, are correlated with host receptor binding and viral persistence. It demonstrates that BeAn virus binds to the host cell receptor in a different mode as GDVII does and, therefore, presents a different phenotype [6]. The ability to bind to sialyloligosaccharides has also been implicated in the phenotypic differences of other viruses. In the study of murine polyomavirus tumor induction in mice, it was found that a single amino acid in its VP1 protein (Glu 91) could influence the profile of tumor growth and was shown to be involved in receptor recognition. Glu 91 is part of the attachment site for sialyllactose revealed by X-ray crystallography [7].

Progress

Although our current data suggested that sialyloligosaccharides are the receptor for the less virulent group of Theiler's virus, it will require structure determination of Theiler's virus complexed with the polysaccharide to clearly define the interaction of virus with its receptor. Not only sialyloligosaccharides are a unique group of receptors for picornavirus, they also provided the first opportunity to study the virus-receptor complex structure at high resolution. All current studies of picornavirus' interaction with their receptors are on the Em level because of difficulties in obtaining suitable complex crystals. In our case, we only need to soak the receptor molecules into the native crystal of the persistent strain of BeAn virus.

The data collected recently using SSRL beamline 7-1 will be collecting diffraction data on TMEV virus crystals soaked in sialyloligosaccharide solutions generated a new density map which showed a potential sialic acid binding site. Further experiments are required to confirm the result.



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